GLUTATHIONE IS RELEASED DURING REPERFUSION FROM INFARCTED BUT NOT FROM STUNNED MYOCARDIUM

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Reactive oxygen metabolites may cause injury in stunned (15 min occlusion) and infarcted (90 min) models of regional canine coronary ischemia and reperfusion. We examined whether myocardial release of reduced (GSH) or oxidized (GSSG) glutathione differs in the two models. GSH is an important defense against oxygen metabolites through oxidation to GSSG. GSH and GSSG release is a possible marker of oxygen metabolite injury. Anesthetized dogs underwent 15min (N=5) or 90min (N=5) left anterior descending (LAD) artery ischemia and reperfusion. Plasma from the great cardiac vein (GCV), draining the LAD region, and aorta were assayed for GSH and GSSG (nmol/ml). LAD endocardial blood flow by microspheres during ischemia was similar in both groups.

GSH-GCV	Pre-Ischemia	End-Ischemia	7 min. Reperfusion	30 min. Reperfusion
INFARCTED	7.7±1.1	10.8±2.6	37.4±6.9*	11.0±1.8*
STUNNED	7.0±0.9	6.4±0.3	7.6±0.8	5.8±0.5
GSSG-GCV				
INFARCTED	0.55±0.15	0.92±0.14	1.70±0.29*	0.87±0.27
STUNNED	0.85±0.35	0.89±0.26	0.66±0.18	0.61±0.15

*p<0.05 STUNNED and pre-ischemia

GSH and GSSG in the aorta were unchanged during ischemia-reperfusion in both groups. GSH and GSSG release occurs during reperfusion only from infarcted myocardium. This implies a more subtle oxidative stress in stunned myocardium, and that tissue disruption due to necrosis may be required for myocardial GSH or GSSG release.

GENERATION OF SUPEROXIDE RADICALS AND RELEASE OF ELASTASE BY NEUTROPHILS DURING THROMBOLYSIS

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Alterations in platelet and neutrophil function during the course of coronary artery thrombosis may influence the extent of myocardial ischemic injury. Number of circulating platelets and neutrophils, neutrophil superoxide radical generation and elastase release were measured in 16 dogs with electrically-induced coronary artery thrombus. These measurements were made before (control) and 30 minutes after thrombus formation, and following thrombolysis with tissue plasminogen activator (t-PA). After thrombus formation, platelet counts slightly decreased from 11.6±7.3 to 10.7±7.8 $x10^7$/ml (P-NS), neutrophil counts increased from 4.8±2.0 to 8.7±3.8 $x10^6$/ml (P<0.05) and neutrophil superoxide radical generation increased from 12.4±5.4 to 16.9±7.4 nmoles/10^6cells/10 minutes (P<0.01). Plasma peptide Bß 30-43, measured as an indicator of fibrinogen degradation by neutrophil elastase, was not detectable before or after thrombus formation. Upon thrombolysis with t-PA, platelet counts continued to decrease (7.5±4.3 $x10^7$/ml, P<0.01 vs. control) and neutrophil counts continued to increase (9.5±4.6 $x10^6$/ml, P<0.01 vs. control). Neutrophil superoxide radical generations increased further to 18.2±4.7 nmoles/10^6cells/10 minutes and Bß 30-43 was detected in large amounts (300±345 nmoles/L). These observations on platelet and neutrophil kinetics suggest platelet consumption in the thrombus and release of neutrophils from storage pools in response to myocardial injury. Activation of neutrophils, as indicated by increased superoxide radical generation and release of large amounts of elastase, may relate to reperfusion-related tissue injury.

TIME COURSE OF INFILTRATION AND DISTRIBUTION OF NEUTROPHILS FOLLOWING CORONARY ARTERY REPERFUSION

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Free radical generation during coronary artery reperfusion has been implicated as a cause of myocyte damage. Neutrophils (PMNs) have been suggested as a possible source; however the time course of infiltration and distribution of PMNs in reperfused myocardium has not been well established. To investigate the influence of coronary reperfusion on PMNs we studied hearts from 4 groups of anesthetized rats subjected to: permanent coronary occlusion for 2 hours (n=15) or 3 hours (n=8) or to 1 hour of occlusion followed by reperfusion for 1 hour (n=16) or 2 hours (n=8). Sections from hearts were examined microscopically (~ 750 fields/section). The PMNs were counted and their location was determined. Reperfusion for 1 hour did not increase the number of PMNs compared with 2 hours of permanent occlusion (80±17/section vs 59±16/section, p=ns). However 2 hours of reperfusion increased PMNs dramatically (529±128/section) compared with 1 hour of reperfusion (80±17/section, p<.001) and with 3 hours of permanent occlusion (121±25/section, p<.01). Over time, the distribution of PMNs changed: after 1 hour of reperfusion, PMNs were located primarily in vessels (74±5%), but after 2 hours of reperfusion only 32±6% were in vessels, with most PMNs located within the interstitium or in myocytes. PMNs appeared to enter the tissue via the subepicardial vessels and stream toward the subendocardium. From these data we conclude that initially PMNs are located in vessels, but by 2 hours of reperfusion, they are located mainly in the interstitium and in myocytes. Reperfusion greatly increased the number of PMNs in the heart compared with permanent occlusion, but the increase did not occur within the first hour of reperfusion. It is unlikely that an increase in the number of PMNs during the first hour of reperfusion accounts for "reperfusion injury" due to free radical damage.

EFFECTS OF INTERLEUKIN-2 (T CELL GROWTH FACTOR) UPON MURINE COXSACKIEVIRUS B3 MYOCARDITIS.

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We have already shown that the development for severe coxsackievirus B3(CB3) myocarditis is regulated not by B cells but by T cells(Circulation 77,645,1988 and 79,1300, 1989). Interleukin-2(IL-2; T cell growth factor) is a T cell-derived lymphokine that stimulates the growth of T cells. Therefore, to test the effects of IL-2 upon CB3 infected BALB/c mice, recombinant IL-2, $5x10^4$U, was administered subcutaneously daily starting on day 0(Grp 2,Exp.I) or day 7(Grp 4,Exp.II) for 7 days, respectively. Splenic natural killer(NK) cell activity was examined by ^{51}Cr-release assay on day 7 or 10. Grps 1 and 3 were infected controls.

	Grp	Survivors (n)		Myocard. Lesion Score(1+ to 4+) INF	N	NK (% Cytotoxicity)
Exp.I	1	30	10	2.1±0.9	1.7±0.6	30.4±7.2 (n=8)
	2	30	24*	1.4±0.7*	0.9±0.6*	70.7±19.1*(n=8)
Exp.II	3	18	10	2.1±0.7	1.9±0.5	9.4±13.3 (n=4)
	4	30	2*	2.6±0.8*	2.5±0.3*	16.1±9.1 (n=4)

*p<0.05 vs Grp 1 or 3, INF=infiltration, (Mean±S.D.)
N=necrosis, NK; against YAC-1 cells(Effector/Target=50). In Exp.I, myocardial virus titers in Grp 2 on day 5(2.0±2.0$x10^2$ plaque-forming units/mg,n=5) were lower(p<0.05) than in Grp 1(8.5±5.5$x10^2$,n=5). In conclusion, IL-2 has the potency to limit myocardial virus by enhancing NK activity in the viremic stage, resulting in reduction of cardiac pathology. On the contrary, it aggravates the course and the severity of the disease in the aviremic stage, probably by T cell activation.